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Eco-ethological analysis of the interaction between isoprene and the behaviour of Collembola

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Summary. Evidence from many sources indicates that the rate of decomposition can be related to the chemical-physical characteristics of the leaves, such as their thickness and C and N content. Whereas some data indicates that Collembola show clear feeding preferences, little is known about the substances responsible for Collembola feeding preferences. In this paper preliminary data on the avoidance behaviour of *Sinella coeca* towards isoprene, an important secondary plant metabolite, are reported. The animals demonstrated a clear avoidance reaction even at concentrations of 10^{-1} μM; despite isoprene volatility, observations made over two consecutive days demonstrated that the clustering of specimens on the food without isoprene persisted over time, probably in relation to the release of aggregation pheromones that stabilize the initial choice. The evident avoidance behaviour shown by *Sinella coeca* warrants further studies in order to describe the defensive potential of isoprene to a broad spectrum of herbivores. The possibility that isoprene may affect litter decomposition rates is discussed.

Key words: Collembola, *Sinella coeca*, secondary plant metabolite, litter decomposition, feeding behaviour, isoprene

Introduction

The role played by Collembola in plant material decomposition is widely recognised (Rusek 1989). Collembola populations are involved in both epigeic and ipogeic plant material breakdown (Stabaeva 1989). The influence of different trophic groups on decomposition rates vary greatly according to plant species and environmental conditions. The relevance of Collembola in plant litter decomposition has led to a lot of research on their diet. The main approaches applied in these studies have been gut content analysis (Lupetti et al. 1989) and food preference experiments. The preference tests have dealt mainly with feeding reactions to bacteria and fungi, but little data are available about plant materials (Massoud & Najt 1976; Rusek 1989). Characteristics of plant material may influence Collembola behaviour indirectly, through affecting microflora colonisation, or directly depending on their thickness and C and N content (Verhoef et al. 1988). A recent hypothesis suggests that secondary plant

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metabolites could affect decomposition rate, as a consequence of their influence on arthropods feeding behaviour (Lambers 1993). Nevertheless, little is known about release of plant metabolites and about their influence on soil fauna.
Isoprene has a 5-C hydrocarbon structure and the isoprene structure is the building block of terpenoids. Isoprene production is linked to photosynthetic activity (Sanadze 1990) and its emission is usually about 2 % of the fixed carbon (Monson & Fall 1989; Loreto & Sharkey 1990). However, it can constitute as much as 50 % of the fixed C under conditions of high temperatures or water stress (Sharkey & Loreto 1993). Although isoprene emission from plants was reported more than 30 years ago (Sanadze 1957), its role remains unclear. It has been hypothesized that isoprene may act as a possible futile pathway for dealing with excess reduced C (Johnson et al. 1997). Recently, its role in thermal protection, particularly in response to rapid changes in leaf temperature, has also been hypothesized (Sharkey & Singsaas 1995). Several isoprenoids, such as monoterpenes and sesquiterpenes, act as a chemical barrier against pests and pathogens (Raffa & Smalley 1995). Some of these compounds are also known to act as allelopathic agents (Whittaker & Feeny 1971). Since the hypothesis of a similar role for isoprene has never been tested, the aim of the research was to study the behaviour of *Sinella coeca* (Schött 1896) toward isoprene.

Materials and Methods

The experimental device (Fig. 1) is similar to that successfully used in previous research on the feeding choices of this species. It was tested using zero treatments; it was proved to be reliable, as the specimens' choice was not affected by factors other than the added compound (Tosi et al. 1977). To simulate leaf surface, two discs of absorbent paper with a surface area of 30 mm^2 were placed in two holes. In one hole the paper was wetted with 2 µl of distilled water, in the other with 2 µl of isoprene water solution. This quantity was chosen in order to wet the paper avoiding the diffusion of isoprene in the device. A small amount of bread crumbs was placed on both discs. Two hours after the introduction of 12 adults into the central part of the device, the number of the specimens in each hole was recorded. Observations (two min) were repeated at least two times every two hours using a cold light binocular microscope. The first experimental series (9 trials, 4 observations each trial) aimed to elucidate the reaction toward different isoprene concentrations, 10^{-3}, 10^{-2} and 10^{-1} µM, and the persistence of the initial choice during two subsequent days. Due to isoprene volatility, the second experimental series (6 trials, 3 observations each trial) aimed to evaluate the disappearance of the avoiding reaction by introducing the specimens 60, 90 and 120 minutes after the enrichment of one of the two holes with 10^{-1} µM isoprene solution. The trials were carried out at 15 ± 1 °C and 60 ± 0.5 % humidity. The significance of the differences in the choices between control and one concentration of isoprene and between the first and the second day was tested by Wilcoxon Matched Pairs Test.

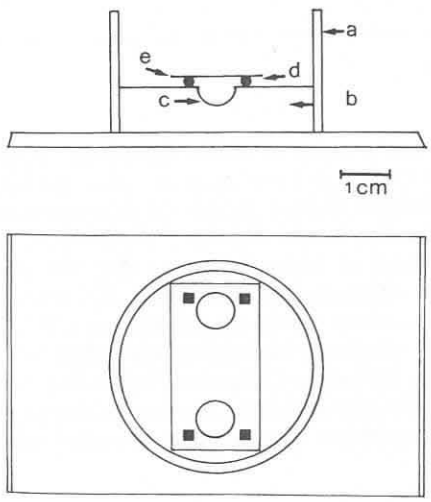

Fig. 1. Diagrammatic sketch of experimental device, a = plexiglass cylinder, b = plaster, c = holes, d = cover-slip supports, e = cover-slip

Results

The specimens of *S. coeca* showed a clear avoidance reaction to isoprene even at concentrations of 10^{-1} µM. This reaction was not detected when concentrations lower than 10^{-2} µM were used. Despite isoprene volatility, observations made over two consecutive days demonstrated that the clustering of the specimens on the food without isoprene persisted over time (Table 1). This may have been because aggregation pheromones stabilized the initial choice. This hypothesis was confirmed by the second experimental series demonstrating that, under the temperature and humidity conditions used, the avoidance reaction eliced by isoprene disappeared after two hours (Table 2).

Tab. 1. Feeding choices of *S. coeca* between the same food with or without addition of isoprene. For each concentration total number of specimens (n), mean number and standard deviation recorded in the 1st and 2nd day are reported. T = number of trials, N = number of records. For further explanations see Materials and Methods.

	10^{-3} µmol				*10^{-2} µmol*			
	1st day		*2nd day*		*1st day*		*2nd day*	
	n	*n ± sd*	*n*	*n ± sd*	*n*	*n ± sd*	*n*	*n ± sd*
isoprene	34	1.9±2.05	39	2.2±2.23	12	0.7±1.02	9	0.6±0.89
control	49	2.7±2.54	47	2.6±2.75	38	2.1±1.67	51	3.2±2.00
	T=9; N=18; P n.s.		*T=9; N=18; P n.s.*		*T=9; N=18; P<0.05*		*T=9; N=18; P<.01*	

	10^{-1} µmol			
	1st day		*2nd day*	
	n	*n ± sd*	*n*	*n ± sd*
isoprene	12	0.7±1.03	7	0.4±0.61
control	90	5.0±2.22	87	4.8±1.85
	T=9; N=18; P<.01		*T=9; N=18; P<.01*	

Tab. 2. Feeding choices of *S. coeca* between the same food without isoprene or added with isoprene solution 1 µmol 60, 90, 120 min before the introduction of the specimens. For further explanations see Tab.1.

	60 min		90 min		120 min	
	n	*n ± sd*	*n*	*n ± sd*	*n*	*n ± sd*
isoprene	10	0.5 ± 0.86	13	0.2 ± 1.41	8	1.1 ± 1.73
control	25	1.4 ± 1.50	52	2.9 ± 1.74	13	0.7 ± 0.65
	T = 6; N = 18; P < 0.05		*T = 6; N = 18; P < 0.05*		*T = 6; N = 12; P n.s.*	

Discussion

Isoprenoid compounds are known to be insecticidal, fungistatic or repellent for herbivores (Pickett 1991), and some fungal metabolites are reported to be toxic for Collembola (Sabatini & Innocenti 1995). Most of the research on isoprene has dealt with its production in leaves in relation to photosynthetic rate and environmental parameters (Loreto & Sharkey 1990), whereas fluxes of isoprene in canopy airspace are far from being fully understood (Seufert et al. 1995). The quantity and distribution of isoprene in soil are unknown, but isoprene has been shown to be released by some mycorrhizal species (Pinetti A., Univ. of Modena, pers. com.). Isoprene emission rates are dependent on the plant species and the environmental conditions. Although the amount of isoprene stored in leaves is reported to be negligible (Monson et al. 1995), the strong avoiding reaction of *S. coeca* to isoprene suggests that isoprene can act as a defense of leaf apparatus. Not all plant species emit isoprene and this variability may represent a selective advantage. However, it is well known that Collembola utilize plant remains as a food source some time after leaf fall. Microarthropod colonisation depends on previous colonisation by micro-organisms (Reisinger & Kilbertus 1975), but also on the release of metabolic compounds from the litter itself. Previous research demonstrated that rinsing with water or alcohol increases the palatability of the bark of some plant species, but the substances responsible for the "litter effect" were not identified (Tosi et al. 1977).
The data suggest that secondary plant metabolites are very important in modulating the decomposition processes. It is worth noting that, in spite of isoprene volatility, the gathering of specimens on the isoprene-free food lasts for a period, probably as a consequence of the release of aggregation pheromones that guide and stabilize Collembola populations on the preferred food (Christiansen 1970; Verhoef 1984). This phenomenon could extend the effect of repellent substances. The present study shows that further research on the feeding behaviour of Collembola in relation to secondary plant metabolites may provide new useful insights into nutrient cycles in terrestrial ecosystems. The data obtained warrant further studies in order to describe the defensive potential of isoprene to a broad spectrum of herbivores and to understand its biological activity in protecting plant tissues.

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